



## Review Paper

# Biocompatibility of Poly (DL-Lactic Acid/Glycine) Copolymers

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**Abstract:** In this review the authors discuss the polymer chemical, physical and cell biological aspects of poly (DL-lactic acid/glycine) copolymers, both *in vitro* and *in vivo*.

The mechanism and rate of degradation and the degree of foreign body reaction were evaluated as a function of the molecular composition of the (co)polymer, its initial molecular weight and changes in crystallinity.

Data from the literature concerning poly(lactic acid), poly(glycolic acid) and poly(amino acids) are included in this review.

The choice to apply the polymers mentioned was determined by their nature: all are present in the human body as natural residues. Upon degradation, biocompatibility will thus not be impaired.

The authors conclude that the degradation mechanism of poly(lactic acid), poly(glycolic acid) and poly(amino acids) are similar, i.e. bulk hydrolysis of ester bonds. The initial molecular weight and the chemical composition, determine the rate of degradation and the degree of foreign body reaction.

## 1 INTRODUCTION

The need for temporary therapeutic aids in the medical field, for example, absorbable implants<sup>1</sup> and drug carrier systems<sup>2</sup> has stimulated the application of degradable polymers. Upon implantation of such materials, the human body reacts by encapsulation or extrusion of this foreign body. The intensity and extent of this reaction varies widely depending on the physical and chemical nature of the foreign body. The combination of physical and chemical factors determines whether the material can be described as 'compatible'.

Biocompatibility is defined as 'the ability of a material to perform with an appropriate host response in a specific application'.<sup>3</sup> This definition covers all eventualities but it is not practical. We therefore prefer the concept that a material of optimal biocompatibility does not lead to an additional acute or chronic inflammatory response

and does not prevent a proper differentiation of implant-surrounding tissue. This implies that every biomaterial will have its own specific requirements for an optimal biocompatibility. Biocompatibility will thus depend on the ultimate application of a material.

In general, a biomaterial can evoke a series of events after its implantation in the body, including

- wound healing reaction<sup>4,5</sup>
- foreign body reaction<sup>6</sup>
- tissue reactions caused by toxic or irritant monomer leachables, plasticizers, hardeners, initiators or fillers<sup>7</sup>
- infection, etc.<sup>8</sup>

Some of these events can be avoided by careful choice of material and implantation procedure. Others, however, will always take place and are influenced by factors such as:

- *Biodegradability*: fragmentation and solubil-

ization of a material will, in general, cause an inflammatory response.<sup>9-13</sup> The degradation products can be toxic, acid or may evoke a physiological response in another way. Additionally, fragmentation products may cause mechanical irritation of the surrounding tissue.

- *Porosity*: the pore size determines whether cells can invade a material and to what extent the foreign body reaction takes place.<sup>14-16</sup>
- *Shape*: the shape and size of a biomaterial is determined by its purpose. From a cell-biological point of view, however, a tube-shaped material<sup>17</sup> with a diameter less than 1 mm<sup>18</sup> will elicit a minimal foreign body reaction in a subcutaneous implantation model. Sharp protrusions or extreme roughness at the surface will cause a more severe fibrous reaction and a prolonged inflammatory response.
- *Physico-chemical nature of the material*: many parameters are involved (e.g. chemical structure, surface charge, wettability, compliance). The first interactions of tissue cells or proteins with a biomaterial will primarily be influenced by these parameters.<sup>19-23</sup>

In many cases, it is difficult to differentiate between tissue reactions as a result of normal wound healing phenomena and as a result of the presence of the foreign body, especially during the first two weeks after implantations. Some factors will even determine part of the wound healing reaction (wettability, mobility, shape, size, porosity, etc.) while others tend to determine the foreign body reaction at a later stage (degradation, compliance).

It is obvious that the biocompatibility of degradable materials is, in general, not as optimal as non-degradable materials. One might then ask why degradable material should be used.

First, the ultimate purpose of the biomaterial might be of a temporary nature, e.g. bone plates, sutures, drug delivery devices, in which it is preferable that the material degrades rather than remains *in situ*. Second, the choice of material can be such that the body does not recognize the implant as foreign, from a chemical point of view. It is possible, for example, to use 'natural' compounds to synthesise a degradable biomaterial. The use of L- and D-lactic acid,<sup>24-28</sup> butyric acid,<sup>29</sup> glycolic acid,<sup>30</sup>  $\alpha$ -amino acids,<sup>31-34</sup> etc., is a logical choice in this context, although it is still difficult to optimize

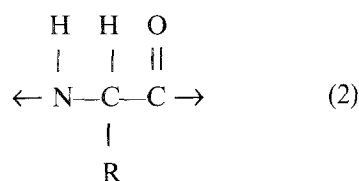
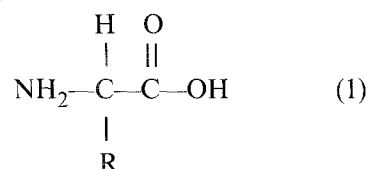
all relevant properties. With regard to biocompatibility, these materials will score high. In the author's laboratories they have experience with the synthesis and cell-biological evaluation of poly L-lactic acids in general. In this overview the biocompatibility of poly (DL-lactic acid/glycine) copolymers will be evaluated and compared with DL-lactic acid/glycolic acid copolymers.

## 2 POLY DL-LACTIC ACID/GLYCIN COPOLYMERS

### 2.1 Introduction

The essence of biodegradable materials is the presence of labile bonds which can be cleaved by hydrolysis<sup>24</sup> or enzymatic activity. Two major classes of degradable polymers that fulfil the above-mentioned prerequisites are: poly ( $\alpha$ -amino acids)<sup>34</sup> and polyesters.<sup>25</sup>

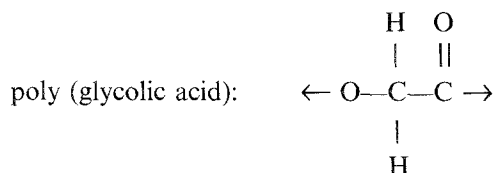
$\alpha$ -Amino acids and poly- $\alpha$ -amino acids can be represented by the general formulas 1 and 2 respectively:

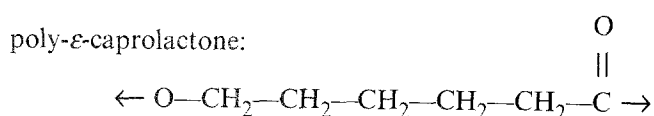
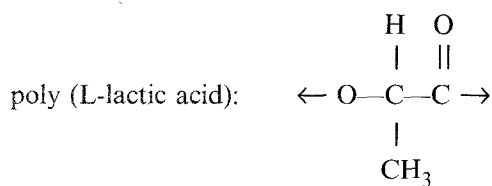


If R = H,	glycine, polyglycine
R = CH <sub>3</sub>	alanine, polyalanine
R = CH(CH <sub>3</sub> ) <sub>2</sub>	valine, polyvaline
R = CH <sub>2</sub> CH <sub>2</sub> -COOH	glutamic acid, polyglutamic acid

The degradation mechanisms of several water soluble poly ( $\alpha$ -amino acids) like L-leucine, L-aspartic acid or glutamic acid and ethyl glutamate are related to the copolymer composition and in particular to the hydrophobicity of the material.<sup>32,35,36</sup>

The chemical structure of a number of well-known degradable polyesters are:





Polyesters generally have been synthesized by ring-opening polymerization of the corresponding (di)lactones.<sup>25</sup> Copolyesters, composed of glycolic acid and L-lactic acid units<sup>25,37,38</sup> or DL-lactic acid units<sup>39,40</sup> have been studied as well as copolymers comprising combinations of  $\epsilon$ -caprolactone and L-lactic acid,<sup>41</sup> glycolic acid<sup>42,43</sup> or DL-lactic acid residues.<sup>40,44</sup>

The degradation mechanism of polyesters has been described as bulk hydrolysis of the ester-bonds.<sup>24</sup> Polyglycolic acid, poly-L-lactic acid and polycaprolactone demonstrate initial hydrolysis in the amorphous regions. In a later stage the crystalline regions are degraded.<sup>45-48</sup> During the initial hydrolysis the overall crystallinity may increase. The total degradation time however is relatively long: between 1 and 2 years. Enzymatic degradation of polymers has been demonstrated both *in vitro*<sup>45,46</sup> and *in vivo*. *In vivo*, enzymes may be involved in the degradation process of the initial hydrolysis products.<sup>47,48</sup>

The development of new degradable materials is undertaken to obtain a choice of materials having

different degradation times, permeability and mechanical properties. Although the properties of the homopolymers can be modified to a certain extent by varying molecular weight and/or crystallinity, copolymerization provides the possibility to prepare materials with a wide range of properties, which depend on the composition.

An interesting class of biodegradable polymers are the polydepsipeptides, polymers composed of  $\alpha$ -hydroxy acid and  $\alpha$ -amino acid residues. Because different amino acids and hydroxy acids can be incorporated in the polymer backbone, a wide variation in polymer structures and properties becomes possible. The synthesis of polydepsipeptides was studied by Goodman and coworkers.<sup>49-53</sup> Copolymers of L-lactic acid and L-alanine,<sup>49-52</sup> D-alanine,<sup>50</sup> L-valine,<sup>53</sup> L-glutamine<sup>51,53</sup> and L-lysine<sup>51,53</sup> have been synthesized on a small scale by thermal polymerization of trifluoro acetate salts of pentachlorophenyl esters of tri- or tetradepsipeptides in the bulk or of the hydrochloride salts in solution. However, the synthesis of polydepsipeptides based on the multistep synthesis procedures described above is inadequate for the preparation of large amounts of polymer. It may be anticipated that ring opening polymerization of 3- and/or 6-substituted 2,5-morpholinediones (cyclic  $\alpha$ -amino acid,  $\alpha$ -hydroxy acid) could be used to prepare alternating polydepsipeptides in a similar way as used in the synthesis of polylactide or polyglycolide.<sup>54</sup>

More recently, the synthesis and properties of several alternating polydepsipeptides, synthesized

**Table 1.** Characteristics of Copolymers Tested *In Vitro*

Code	F <sub>1</sub>	F <sub>2</sub>	M <sub>n, app</sub>	M <sub>w, app</sub>	T <sub>g</sub> (°C)	M <sub>n,c</sub>
0	0	0.5	6000	10000	109	—
25	0.25	0.64	19000	24000	86	—
50	0.50	0.77	5000	15000	75	39000
60	0.60	0.82	11000	30000	69	27000
70	0.70	0.87	20000	43000	66	32000
80	0.80	0.91	38000	67000	61	65000
90	0.90	0.96	48000	97000	58	40000
100	1.00	1.00	167000	426000	54	142000

F<sub>1</sub> represents the mole fractions of DL-lactide in the feed.

F<sub>2</sub> represents the mole fractions of DL-lactic acid residues in the copolymer.

Copolymers 0 and 25 were copolymerized at a monomer/initiator ratio of M/I = 2500 for 33 h.

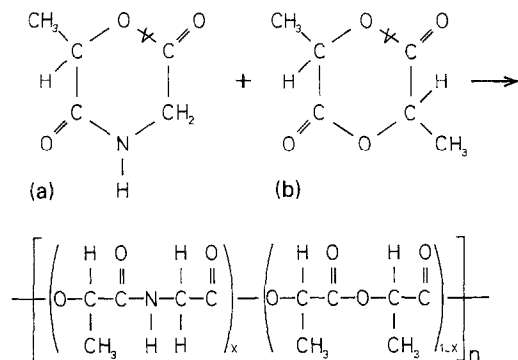
Copolymers 50–100 were copolymerized at M/I = 2500 for 48 h.

M<sub>n, app</sub> and M<sub>w, app</sub> were determined for T<sub>1</sub> and T<sub>2</sub> in DMF (GPC/LALLS).

For 50–100, THF was used in gel permeation chromatography.

T<sub>g</sub> was determined with DSC.

M<sub>n,c</sub> represents the calculated molecular weight by multiplying M<sub>n, app</sub> with M<sub>n</sub>/M<sub>n, app</sub>, the calculated values of M<sub>n</sub> give a better estimation of the real molecular weight values.



**Fig. 1.** Synthesis of glycine/DL-lactic acid copolymers in which  $x$  represents the molar ratio of cyclo (glycine/DL-lactic acid) and  $1-x$  represents the molar ratio of DL-lactide.

via ring opening polymerization of various 3- and/or 6-substituted 2,5-morpholinediones, has been described.

Degradation times *in vivo* of polydepsipeptides range from several weeks<sup>55,56</sup> to several months.<sup>55,57,58</sup> There are indications that poly-esteramides can be degraded *in vivo* by simple hydrolysis of ester bonds.<sup>59</sup>

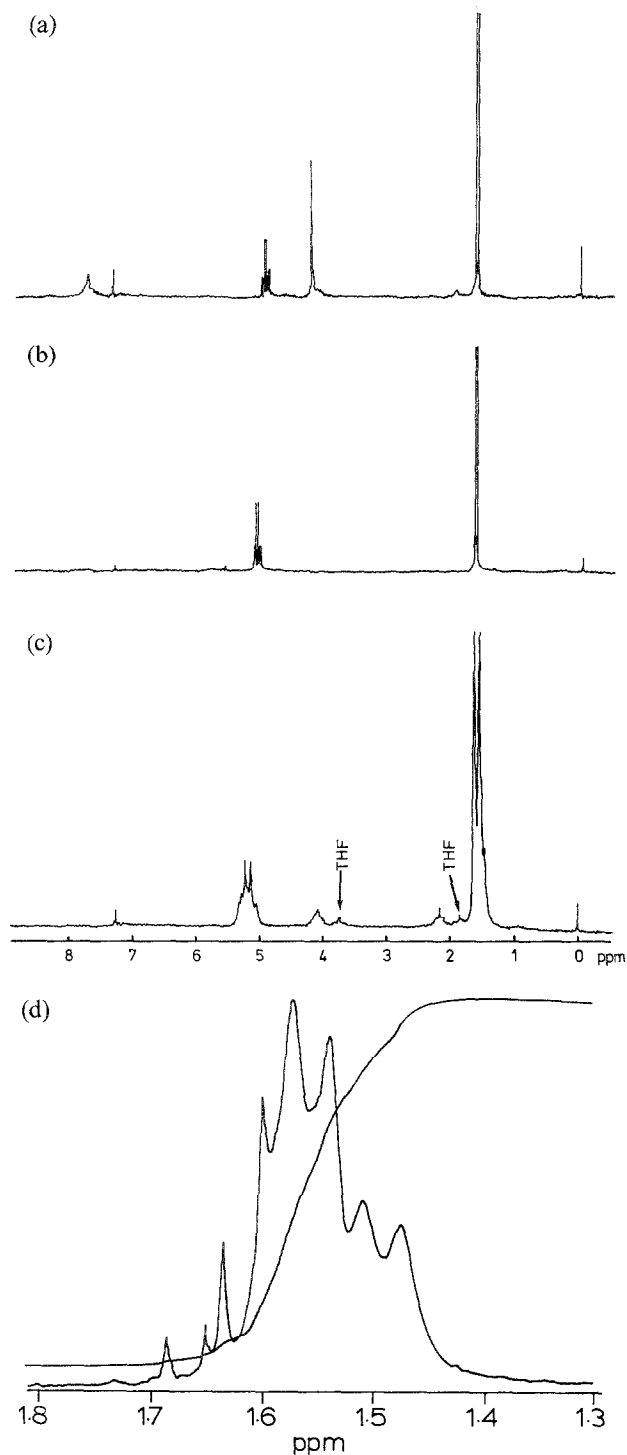
## 2.2 Synthesis and characterization

(DL-lactic acid/glycin) copolymers were synthesized (see Fig. 1) by ring-opening copolymerization in the bulk of cyclo (glycine/DL-lactic acid) (Fig. 1(a)) and DL-lactide (Fig. 1(b)). The initiator in this polymerization reaction was stannous octoate (tin (II) bis (2-ethylhexanoate), a preferred initiator in the preparation of high molecular weight polyesters. Optimization of the reaction conditions revealed that the polymerization was best performed at 130°C, during 48 h and using a monomer to initiator ratio of 2500.

The reaction was described in detail by Helder *et al.*;<sup>54</sup> several copolymers were synthesized by this method, having different molar ratios of DL-lactic acid and glycine residues. The copolymers were characterized by their molecular weight, molar composition and glass transition temperature, as demonstrated in Tables 1 and 2. The crude

**Table 2.** Water- and  $\alpha$ -Bromonaphthalene Contact Angles (degrees), the Polar Component of the Surface Free Energy ( $\gamma_p$ ) and Total ( $\gamma_s$ ) Surface Free Energy (erg. cm<sup>-2</sup>)

Code	$\theta_{\text{H}_2\text{O}}$	$\theta_{\alpha\text{-Br}}$	$\gamma_p$	$\gamma_s$
60	70	30	24.2	62.8
75	75	30	12.2	50.8
80	75	25	13.2	53.5
100	80	20	12.3	54.1



**Fig. 2.** <sup>1</sup>H-NMR spectra of (a) pure monomer DL-lactide, (b) cyclo (glycine/DL-lactic acid), (c) purified copolymer 60, and (d) the spectrum of crude glycine/DL-lactic acid copolymer.

polydepsipeptides were purified by dissolving the polymerization products in THF or chloroform and subsequent precipitation in ethanol. The solvent used in the purification of poly(glycine, DL-lactic acid) was DMF.

The monomer conversion and molecular composition of the copolymers was determined using <sup>1</sup>H-NMR spectroscopy, using CDCl<sub>3</sub> or DMSO-d<sub>6</sub>

(poly{glycine-DL-lactic acid}) as solvent and tetramethylsilane (TMS) as an internal reference. The spectra were recorded with a Nicolet 200 MHz NMR apparatus. The composition was calculated from the methylene and methine proton integrations. The glycine is represented by the methylene proton signal at 4.0 ppm. The signal at 5.1 ppm results from both monomeric units.

Figure 2 shows NMR spectra of (a) the pure monomer DL-lactide, (b) cyclo (glycine-DL-lactic acid), and (c) purified copolymer 60. As can be observed, the doublets of the methyl protons of both monomers are found somewhat more downfield than those of the copolymer. To obtain the conversion of 6-methylmorpholine-2,5-dione and DL-lactide, residual amounts of the monomers in the crude polymerization products were determined from the NMR-spectra expanded in the methyl proton region (Fig. 2(d)). The monomer conversion was determined from the relative intensities of the methyl proton doublets of the monomers and copolymer. The overall composition was calculated from the methylene proton integrations.

The *molecular weight* was determined using GPC or GPC/LALLS as already mentioned.<sup>54,60</sup> The GPC curves of the copolymerization product showed that the molecular weight distribution becomes broader at longer reaction times. This broadening was attributed to an increase in  $M_w$  with constant  $M_n$  due to the occurrence of ester interchange reactions.<sup>61</sup> Other factors such as the difference in the rate of conversion of both monomers and the different elution volumes of chains of different compositions also may be important. The composition of the copolymers was therefore analysed during the polymerization reaction. Spectroscopic analysis showed that the mole fraction of DL-lactic acid/DL-lactic acid residues (from the incorporation of DL-lactide) in the copolymer is slightly higher than that of glycine/DL-lactic acid residues during the initial stages of the reaction (see also Table 1). The rate of incorporation of DL-lactide in the copolymers is higher than that of morpholinedione.

This means that a drift in monomer feed composition with conversion occurs. Obviously, during the final stages of the polymerization reaction the incorporation of glycine/DL-lactic acid residues increases. Probably, some chains are therefore relatively rich in glycine/DL-lactic acid residues and others are relatively rich in DL-lactic acid/DL-lactic acid residues.

Figure 3 shows the relationship between  $M_w, app$

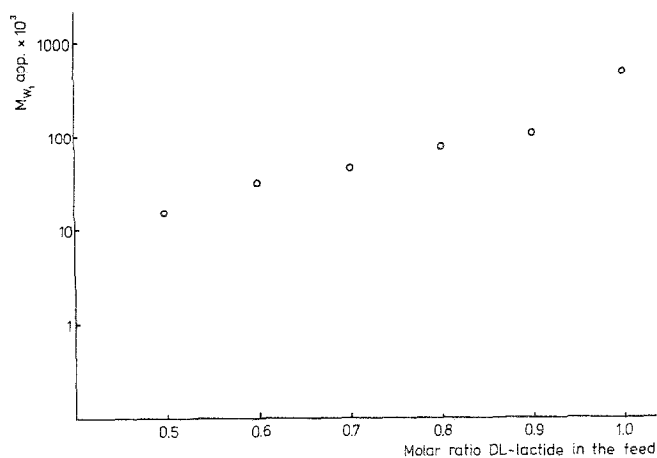


Fig. 3. The relation between  $M_w, app$  and the mole fraction of DL-lactide in the feed. Note that  $M_w, app$  increases with increasing amount of DL-lactide.

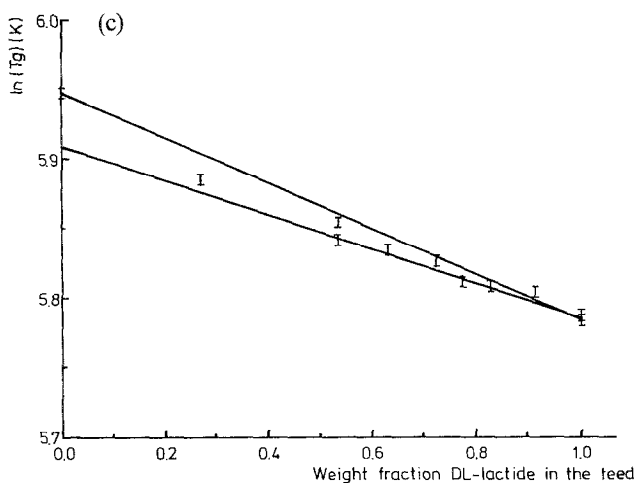
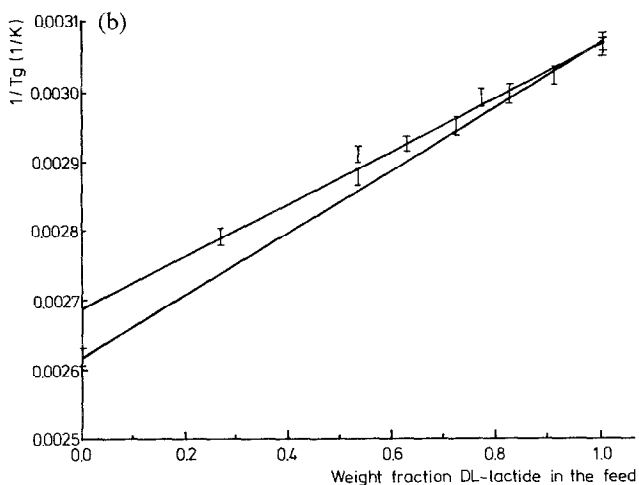
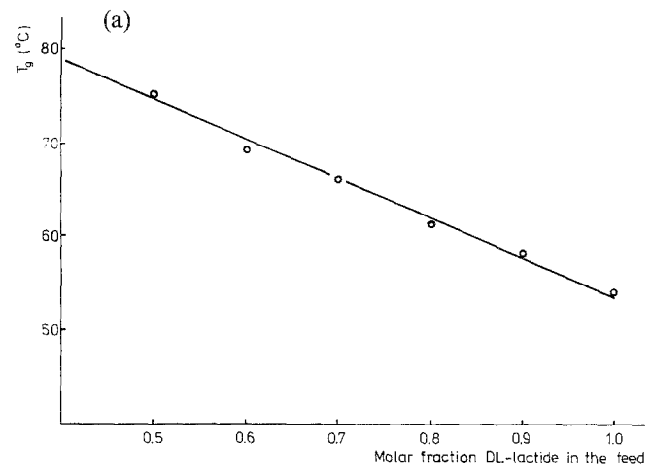
and the mole fraction of DL-lactide in the feed. We can observe that  $M_w, app$  increases with an increasing amount of DL-lactic acid.

Glass transition temperatures ( $T_g$ ) were measured with differential scanning calorimetry (DSC) with a Dupont 990 thermal analyser. In Fig. 4(a) the relation between  $T_g$  and the molar ratio of DL-lactide is given. The glass transition temperatures of the copolymers increase with increasing mole fraction of glycine residues in the copolymer.

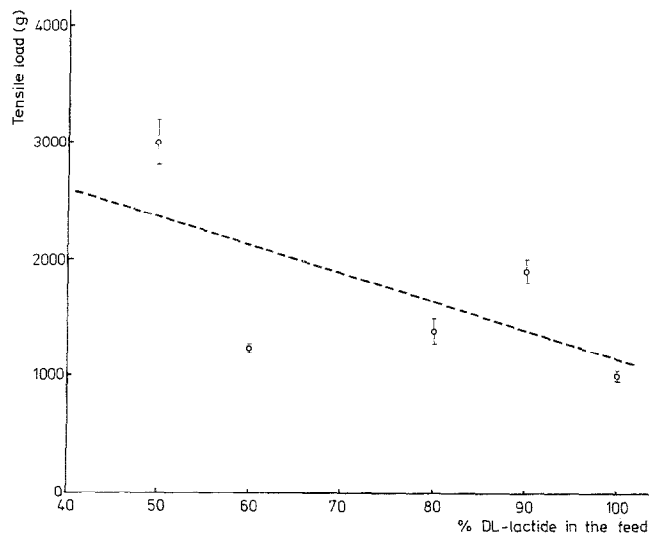
The glass transition temperatures of the glycine/DL-lactic acid copolymers deviate from the lines according to the Fox<sup>62</sup> and Pochan<sup>63</sup> equations (see also Fig. 4(b) and 4(c)). The extent of deviation is larger for the copolymers with increasing glycine content. This deviation must be attributed to the fact that poly(glycine/DL-lactic acid) is an alternating copolymer. The glass transition temperatures of alternating copolymers differ from those of non-alternating copolymers having the same overall composition.<sup>64</sup>

The extent of the deviation depends on the sequences of the components in the non-alternating copolymers and is larger when the alternating sequence is less present in the copolymer. This feature can be explained by proposing that the overall chain flexibility of the copolymer is affected by the introduction of an A—B hetero-link. The effect depends upon whether the rotational barrier of the A—B link is larger or smaller than the average of A—A and B—B homo-links.<sup>64</sup>

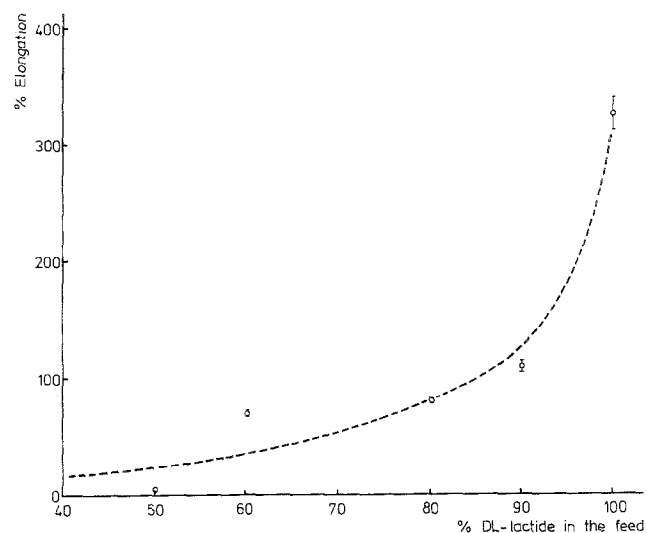
The *tensile load* profile (Fig. 5) shows that in general the strength decreases with increasing DL-lactic acid content. On the other hand, the percentage elongation increases with an increasing



**Fig. 4.** (a) Glass transition temperature ( $T_g$ ) versus the molar ratio of DL-lactide in the feed;  $T_g$  of the copolymer increases with higher glycine content of the copolymer. (b) Comparison of the reciprocal  $T_g$  values of the copolymers with the line according to the Fox equation (lower line). (c) Comparison of the reciprocal  $T_g$  values of the copolymers with the line according to the Pochan equation (upper line).



**Fig. 5.** Tensile load at break versus the percentage of DL-lactide in the feed.



**Fig. 6.** Percentage elongation of the copolymers as a function of the percentage DL-lactide in the feed.

amount of DL-lactic acid residues in the copolymers (Fig. 6).

Scanning electron microscopy reveals the porous nature of the glycine/DL-lactic acid copolymer disks. Figure 7 shows the surface of copolymer 60. The micropores are clearly visible. Figure 8 shows a cross-section through a disk of copolymer 80; two different layers can be observed. A thin porous outer layer (approximately 100  $\mu\text{m}$ ) and a solid inner structure. Other copolymers have similar morphologies though pore sizes and percentage porosity may differ slightly.<sup>60</sup> Mechanical properties of several of the copolymers are shown in Figs 5 and 6.

The surface properties of some copolymers (codes: 60, 75, 80 and 100) were determined by

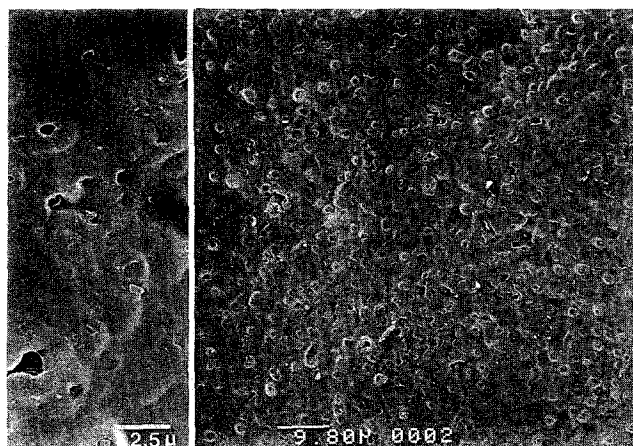


Fig. 7. Scanning electron micrograph of the surface of copolymer 60 (Table 3); the surface is relatively smooth with occasional pores which are smaller than  $1\ \mu\text{m}$  (see insert).

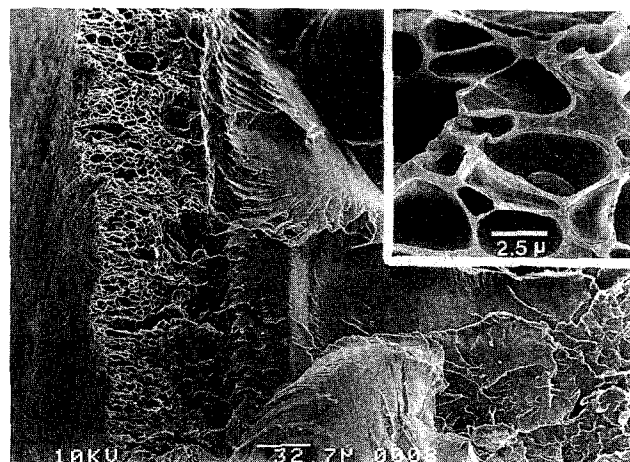


Fig. 8. Scanning electron micrograph of a transection through a disk of copolymer 80. The porous outer layer can be seen in more detail in the insert.

means of *contact angle* measurement, as described in detail by Busscher *et al.*<sup>65</sup> Table 2 shows the water- and  $\alpha$ -bromonaphthalene contact angles (degrees), the polar component of the surface free energy and total surface free energy ( $\text{erg. cm}^{-2}$ ). In general, the more glycine residues are present in the copolymers, the more hydrophilic the surface appears. From recent work<sup>66-68</sup> on the influence of surface free energy on the spreading and growth of cells, it is clear that the more hydrophilic a material, the better cells will adhere and spread (with the exception of very high surface free energy hydrogels). We therefore expect a more pronounced tissue reaction on the copolymers high in glycine content. The presence of leachables in the copolymers might however interfere with these predictions.

In concluding this section it is demonstrated that copolymerization of cyclo(Gly-Lac) and DL-lactide offers a convenient method to obtain glycine/DL-

lactic acid copolymers of varying compositions. The molecular weights of the copolymers are in the same order of magnitude, but are lower than those of PDLA prepared under similar reaction conditions. At low monomer conversions the DL-lactide was preferentially incorporated in the copolymer, eventually resulting in a deviation of random distribution of the monomers in the copolymer.

### 2.3 Degradation

Degradation of the different lactic acid/glycine copolymers was studied as a function of time. Both in-vitro and in-vivo studies were performed.

In the in-vitro studies the copolymers, as presented in Table 1, were cast onto silanized glass surfaces to obtain discs with a diameter of 12 mm, an average thickness of 0.5 mm and an average weight of 50 mg, as described in detail by Helder

Table 3. Three Copolymers Studied Both *In Vitro* and *In Vivo*

Code	F <sub>1</sub>	F <sub>2</sub>	M <sub>n</sub> , app	M <sub>w</sub> , app	T <sub>g</sub> (°C)	M <sub>n,calc</sub>
60	0.6	0.82	6000	18000	69	15000
80	0.8	0.91	19000	39000	61	33000
100	1.0	1.00	24000	52000	54	20000

Polymer 100 (PDLA) is a low molecular weight reference. DL-lactide was kindly provided by CCA, Biochem, Gorinchem, The Netherlands.

F<sub>1</sub> represents the mole fractions of DL-lactide in the feed.

F<sub>2</sub> represents the mole fractions of DL-lactic acid residues in the copolymer.

M<sub>n, app</sub> and M<sub>w, app</sub> were determined for T<sub>1</sub> and T<sub>2</sub> in DMF (GPC/LALLS).

T<sub>g</sub> was determined with DSC.

M<sub>n,calc</sub> represents the calculated molecular weight by multiplying M<sub>n, app</sub> with M<sub>n</sub>/M<sub>n, app</sub>, the calculated values of M<sub>n</sub> give a better estimation of the real molecular weight values.

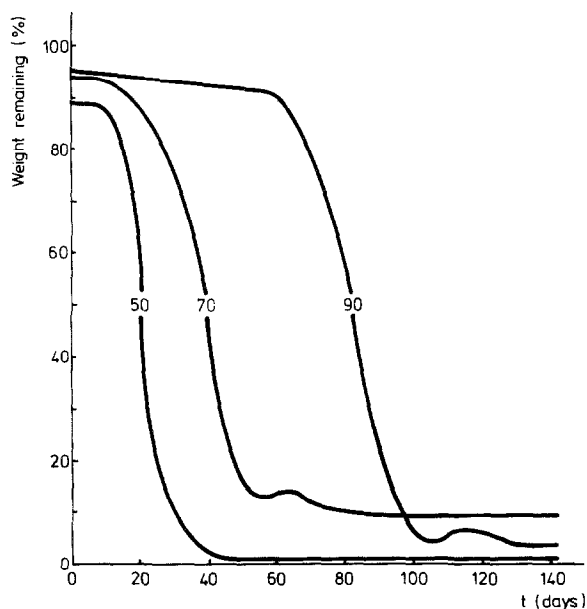


Fig. 9. In-vitro degradation; percentage of mass remaining as a function of immersion time in buffer of copolymers 50, 70 and 90.

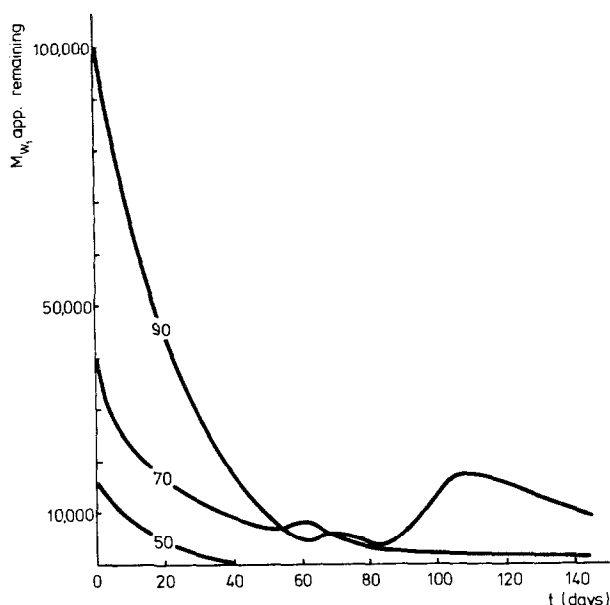


Fig. 10. In-vitro degradation; remaining molecular weight as a function of immersion time in buffer of copolymers 50, 70 and 90.

*et al.*<sup>60</sup> The disks were placed in a buffer solution (0.1 M  $\text{Na}_2\text{HPO}_4$  and  $\text{KH}_2\text{PO}_4$  at pH = 7.4) at 37°C. When the pH dropped below 7.2, the buffer was exchanged. At different time intervals disks were removed from the buffer and mass and molecular weight were determined. In Figs 9 and 10, the mass loss and molecular weight ( $M_{w, app}$ ) loss of polymers 50, 70 and 90 (see Table 1) is illustrated. We observe in Fig. 9 that an initial weight loss occurs in the first few days for all copolymers. This is probably due to the presence of a few per cent of

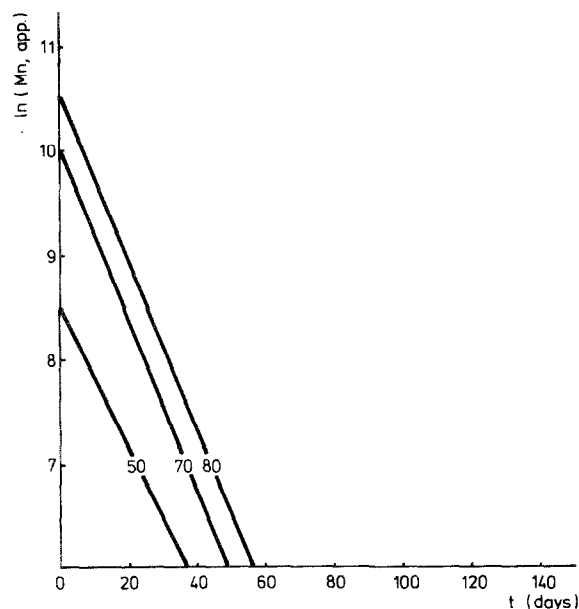


Fig. 11. In-vitro degradation; rate of degradation ( $\ln M_{n, app}$ ) as a function of immersion time in buffer of copolymers 50, 70 and 80.

residual solvent (THF), as can also be seen in the  $^1\text{H-NMR}$  spectrum (Fig. 2(c)). For use in an in-vivo model the disks should be rinsed extensively before implantation, since an adverse reaction might well be due to the presence of solvent rather than incompatibility of the biomaterial.

Copolymer 50 starts losing mass at approximately 10 days, copolymer 70 at approximately 25 days, copolymer 90 at approximately 70 days and pure poly(DL-lactide) at 120 days. For all polymers the time interval in which mass loss takes place is approximately 30 days (steep part of the curves).

Apparently, when the molecular weight reaches a value of approximately 10000 ( $M_{w, app}$ ) the polymers become soluble. Molecular weight loss (Fig. 10) illustrates the above-mentioned mechanism. In Fig. 11 we observe that the rates of degradation, as measured by loss in molecular weight, are similar for all three copolymers. Nevertheless, weight loss occurred sooner for copolymers containing more glycine residues. Probably, the higher solubility of the fractions rich in glycine, which are more hydrophilic than fractions rich in lactic acid residues, is responsible for this phenomenon.

Recently several authors have tried to describe the degradation mechanisms for the hydrolysis of polyesters.<sup>69-72</sup> Two equations<sup>71,72</sup> describe the hydrolysis:

— autocatalysed hydrolysis (by the COOH groups):

$$\ln(M_n) = \ln(M_n^0) - k_1 \cdot t \quad (3)$$



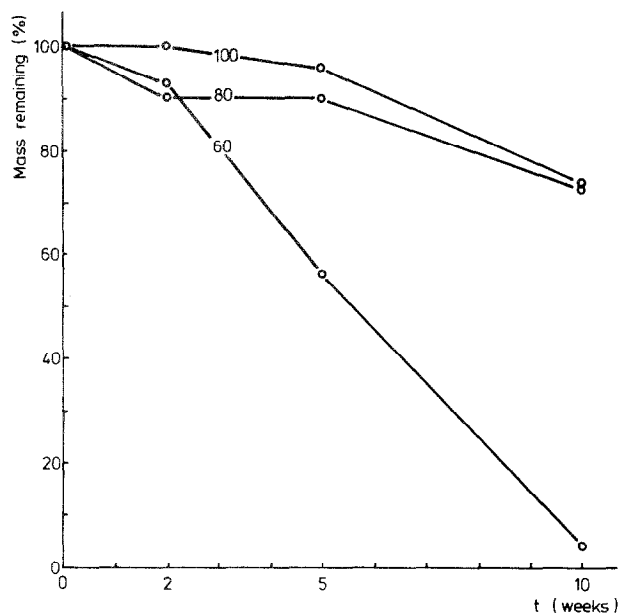


Fig. 12. In-vivo degradation; percentage of mass remaining as a function of implantation time of copolymers 60, 80 and 100.

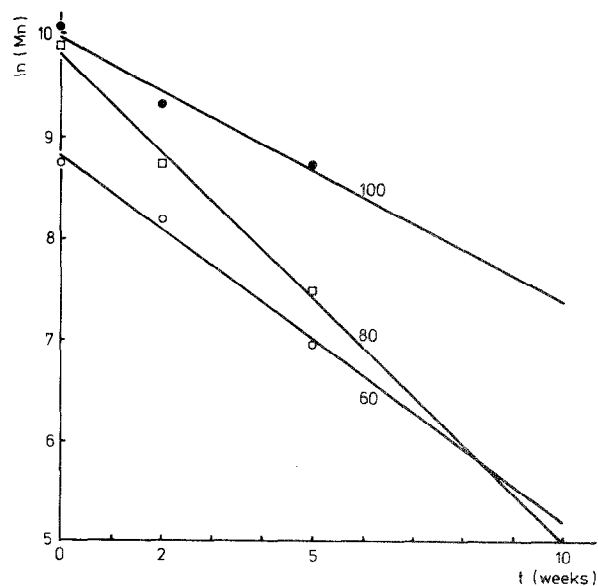


Fig. 14. In-vivo degradation; rate of degradation ( $\ln M_n, app$ ) as a function of implantation time of copolymers 60, 80 and 100.

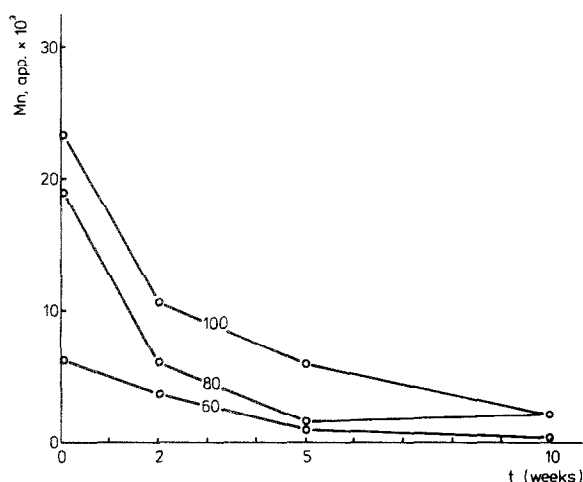


Fig. 13. In-vivo degradation; remaining molecular weight as a function of implantation time of copolymers 60, 80 and 100.

— uncatalysed hydrolysis:

$$1/M_n = 1/M_n^0 + k_2 \cdot t \quad (4)$$

Equation (3) fits the observations as shown in Fig. 11 and it is therefore concluded that the mechanism of degradation of glycine/DL-lactic acid copolymers is consistent with autocatalytic hydrolysis. Hydrolysis of these copolymers presumably mainly occurs by ester bond scission.

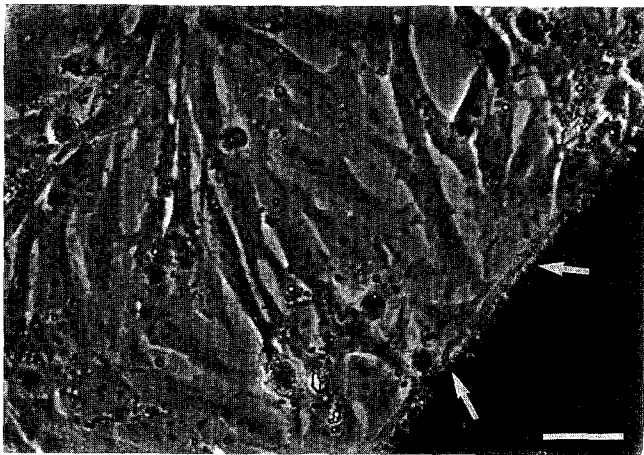
In-vivo degradation of copolymers 60, 80 and 100 was studied by subcutaneous implantation in the rat.<sup>58</sup> The copolymer disks remained implanted for 2, 5 or 10 weeks. In Fig. 12 the mass losses *in vivo* of copolymers 60, 80 and 100 are shown. Figure 13 demonstrates the loss of molecular weight *in vivo* as a function of time.

We can observe similar phenomena as observed in the *in vitro* studies. The weight loss starts as soon as the molecular weight reaches values beneath approximately 5000 ( $M_n, app$ ).

Degradation rates *in vivo* (Fig. 14) are somewhat lower than *in vitro* (Fig. 11). This phenomenon was also observed by Pitt<sup>40</sup> for the degradation of poly(DL-lactide) and poly- $\epsilon$ -(caprolactone). A possible explanation could be the formation of a fibrous tissue capsule around the implant, resulting in an accumulation of degradation products. Furthermore, the proteinaceous environment may result in a lower solubility of the material.

## 2.4 Biocompatibility

Biocompatibility was tested both *in vivo* and *in vitro*. In-vitro tests, using a cell culture system in which the biomaterial is in direct contact with fibroblast cells, showed that the copolymers tested (60, 80 and 100) were biocompatible. Figure 15 shows a cell culture of fibroblasts in the presence of copolymer 60, after 4 days. No halo formation (cell death in the immediate vicinity of the test sample) was observed. Cellular morphology seemed normal as compared to control cultures on tissue culture polystyrene. Cells in contact with the biomaterials did not show signs of impaired function. A well-spread spindle-shaped morphology without the occurrence of spider-like retractions,<sup>73</sup> indicative of decreased adherence, was observed. The growth rate, as established by the cell count, did not



**Fig. 15.** In-vitro biocompatibility test of copolymer 60; direct contact test using human skin fibroblasts. Cell morphology and growth are not impeded by the presence of the biomaterial. Note the close alignment between cells and polymer (arrows). The bar represents 30  $\mu\text{m}$ .

decrease, in comparison with control cultures on tissue culture polystyrene. In both cases the plateau values were reached within 4 days.

Similar in-vitro observations were made for copolymers 80 and 100. Although previous in-vitro degradation studies (NMR) showed that residual solvent (THF) was still present in the copolymer disks (Fig. 2(c)), extensive rinsing in saline solution prior to the tissue culture tests resulted in such a low level of THF that cell growth and morphology were not impaired.

It has been demonstrated many times<sup>74-81</sup> that in-vitro tests are often more sensitive than their in-vivo counterparts in gaining information on biocompatibility and material characteristics. An example of a very sensitive in-vitro test to screen future biomaterials is the gel-culture test, as recently described by van Luyn *et al.*<sup>82</sup>

An intermediate test system between in-vitro and in-vivo tests is offered by the cage implant system as described by Anderson *et al.*<sup>83</sup> A physiological environment is provided without direct contact between phagocytes and the biomaterial tested.

As Coleman *et al.*<sup>84</sup> already stated, in-vivo tests are often too cumbersome for routine evaluation of new materials. Additionally, in-vivo tests are seldom quantitative but remain descriptive and highly subjective. Nevertheless, in-vivo studies are inevitable; questions concerning to what extent mechanical trauma contributes to the reaction, what the most tolerable structural unit size for the implant is; and what part of the overall reaction is caused by surface morphology, etc., remain to be answered.

The in-vivo implantation tests of the lactide/

glycin copolymer disks comprised subcutaneous implantation on the back of rats for 2, 5 and 10 weeks respectively. The results on molecular weight and mass loss have been presented in Figs 12 and 13.

Disks for determination of tissue reactions were fixed in 2% glutaraldehyde.<sup>58</sup> Subsequently samples were prepared for light microscopy by embedding in glycol methacrylate as described in detail by Gerrits *et al.*<sup>85</sup>

After polymerization was completed, sections were cut and stained with toluidine blue and alkaline fuchsin (a specially developed stain for embedded biomaterials).<sup>86</sup>

Light micrographs show that the overall tissue reaction is governed by two main principles

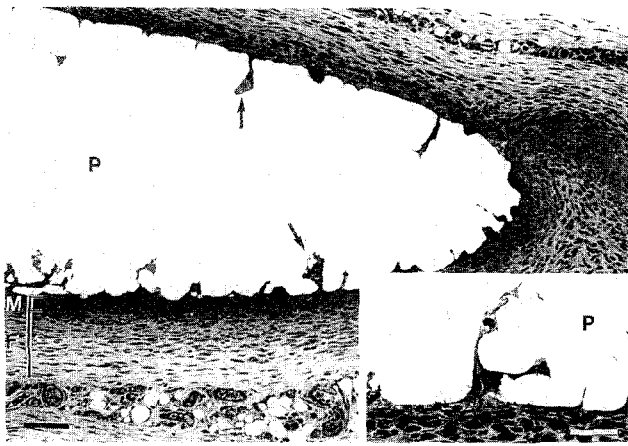
- (1) the general wound healing reaction, which is comparable for all three different implants (60, 80 and 100);
- (2) the physico-chemical nature of the implant.

Wound healing lasts approximately 2 weeks, indicating that at the first time interval (2 weeks) wound healing has been completed and the reactions observed are due to the material properties. The nature of the implant determines further tissue reactions. As shown in Figs 12 and 13, the material becomes soluble at a molecular weight of approximately 5000 ( $M_n$ ). This event is apparently the trigger for an enhanced foreign body reaction: macrophages fusing to giant cells, polymorphonuclear granulocytes, sprouting blood vessels, etc.<sup>87,88</sup>

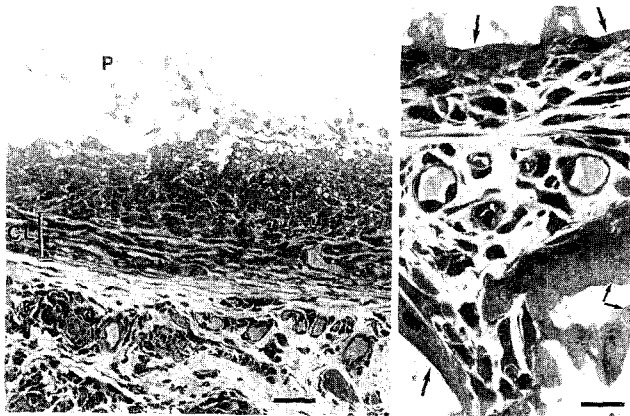
Wound healing reactions, as observed for copolymers 60, 80 and 100, do not differ from a normal wound healing without a biomaterial present:<sup>5,89,90</sup> an initial influx of granulocytes, immediately followed by monocytes, converting into tissue macrophages.<sup>91</sup> After approximately 3 days the acute inflammatory response ends and fibroblasts start migrating towards the healing site (attracted by chemotactic agents produced by macrophages).<sup>92</sup> In a later stage (10 days) these macrophages produce factors that stimulate fibroblasts to synthesize collagen.<sup>58</sup>

For copolymer 60, we can observe from Fig. 13 that within 2 weeks the molecular weight drops beneath the  $M_n, app$  of approximately 5000. Therefore the wound healing reaction around copolymer 60 is immediately followed by a foreign body reaction (Fig. 16).

Macrophages infiltrate the disintegrating matrix of copolymer 60 (arrow). Foreign body giant cells can already be observed. Approximately five layers

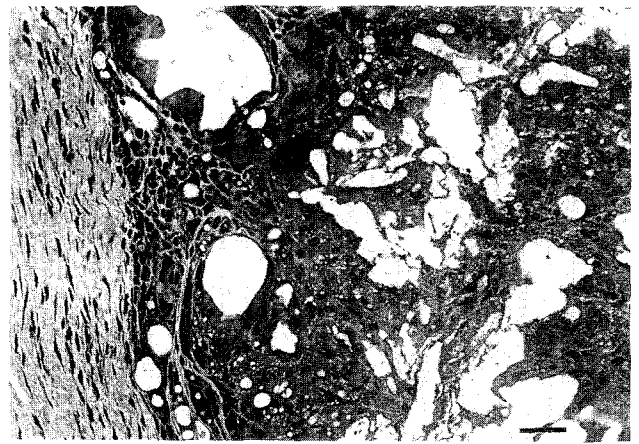


**Fig. 16.** Light micrograph of copolymer 60 (P) after 2 weeks of implantation. Macrophages infiltrate the disintegrating matrix (arrows). Approximately five layers of macrophages (M) and a fibrous layer (F) could be observed around the implant. The bar represents  $100\ \mu\text{m}$ . The macrophages can be seen in more detail in the insert (bar =  $40\ \mu\text{m}$ ).

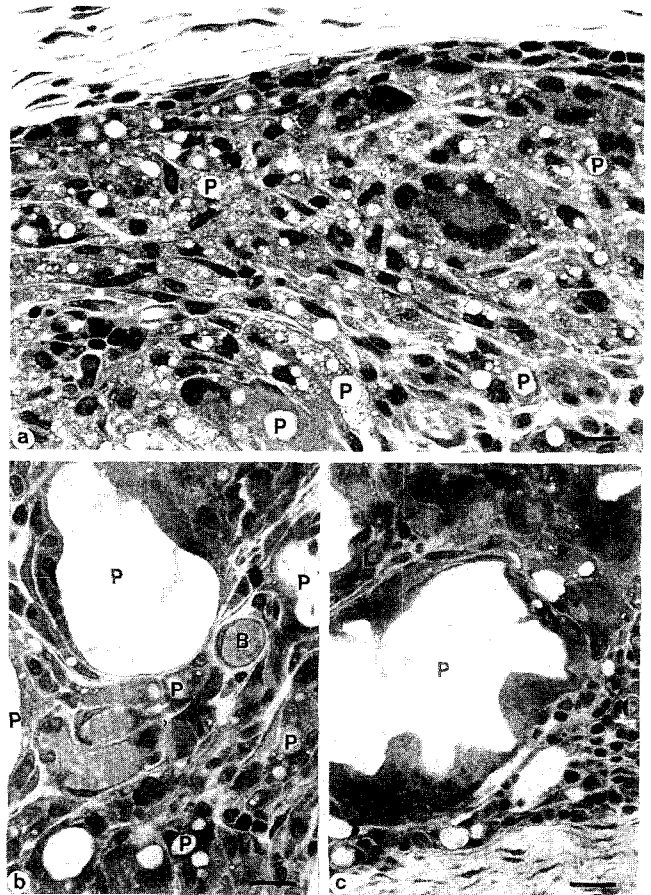


**Fig. 17.** Light micrograph of copolymer 60 (P) after 5 weeks implantation. Blood vessels can be observed within the material (insert). Giant cells and macrophages are positioned directly against the material (arrows). The thickness of the cellular layer outside the implant (CL) did not increase. The bars represent  $63\ \mu\text{m}$  and  $15\ \mu\text{m}$  respectively.

of macrophages (M) were observed directly around copolymer 60. A fibrous layer (F), containing many blood vessels and capillaries had developed around the implant. Deposition of collagen by these fibroblasts could occasionally be observed. After 5 weeks (Fig. 17) 50% of the biomaterial has already degraded. Light micrographs show extensive tissue ingrowth into the biomaterial. Blood vessels (B) can be observed within the material. Large amounts of giant cells and single macrophages are positioned directly against the material in an attempt to engulf the foreign material (arrows). Many parts of polymer debris have already been phagocytized and are degraded intracellularly. The thickness of the cellular layer outside the implant (macrophages and fibroblasts) did not increase in time. The activity is apparently directed inward.



**Fig. 18.** Light micrograph of copolymer 60 after 10 weeks of implantation. The polymer bulk is completely invaded by macrophages, giant cells, blood vessels, etc. Only small fragments of the polymer remain. The bar represents  $40\ \mu\text{m}$ .



**Fig. 19.** Copolymer 60 after 10 weeks of implantation. (a) Polymer fragments (P) can be seen inside macrophages and giant cells; the bar represents  $15\ \mu\text{m}$ . (b) and (c) Large and small polymer particles either surrounded by macrophages and giant cells or internalized for intracellular degradation. Blood vessels and capillaries can be observed throughout the implant. The bars represent  $15\ \mu\text{m}$  and  $25\ \mu\text{m}$  respectively.

Figure 12 shows that, after 10 weeks of implantation, only a few weight per cent remains. This is confirmed by the light micrographs (Fig. 18). Figure 19(a), (b) and (c) show the remaining

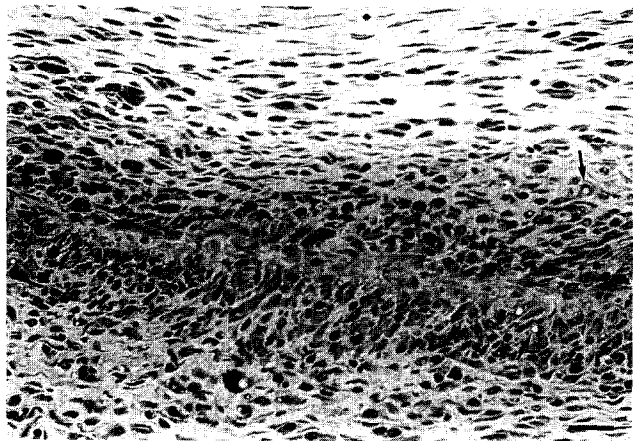


Fig. 20. Copolymer 60 after 16 weeks of implantation. Only organized fibrous tissue remains. Some very small polymer particles can still be seen (arrow). The bar represents 40  $\mu\text{m}$ .



Fig. 22. Copolymer 80 (P) after 5 weeks of implantation. Limited infiltration into the polymer can be observed. The bar represents 63  $\mu\text{m}$ .

polymer particles (P) being degraded by macrophages and giant cells. Blood vessels (B) are observed throughout the implant. The fibrous capsule around the implant has completely matured; the number of fibroblasts has decreased again; no more collagen has been deposited. When all the polymer material had degraded only some scar tissue remained (Fig. 20).

A similar cascade of events was observed for copolymers 80 and 100, except that the original molecular weight ( $M_n$ ) was higher: 19000 for copolymer 80 and 24000 for polymer 100. Since the rate of degradation was similar for all copolymers (Fig. 14), it is inevitable that the  $M_n$ , *app* at which the copolymer fragments become soluble, is only reached several weeks later (see Fig. 14): for copolymer 80, at approximately 3 weeks, and for polymer 100, at approximately 6 weeks.

The light micrographs (Fig. 21) show that after 2

weeks hardly any tissue infiltration in copolymer 80 (P) had occurred. The fibrous layer around the implant was comparable with copolymer 60, although more macrophages seemed to be present at the interface with the material. After 5 weeks, limited infiltration of macrophages and giant cells was observed (Fig. 22), comparable with the situation at two weeks for copolymer 60.

After 10 weeks extensive tissue infiltration in the implant was observed (Fig. 23). This stage was comparable with copolymer 60 at week 5.

Polymer 100 demonstrated hardly any visible degradation until week 10. After 10 weeks only minor phagocytic activity was observed (Fig. 24).

These observations confirm the degradation hypothesis of ester bond scission of the bulk. The higher the molecular weight, the longer it takes for the polymer bulk to reach a molecular weight value, from which the polymer fragments become soluble.

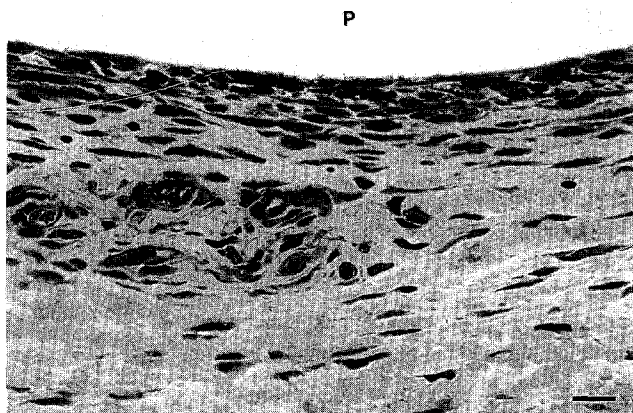


Fig. 21. Copolymer 80 (P) after 2 weeks of implantation. A few layers of macrophages and fibroblasts can be observed around the implant. Hardly any infiltration into copolymer 80 is observed. The bar represents 25  $\mu\text{m}$ .

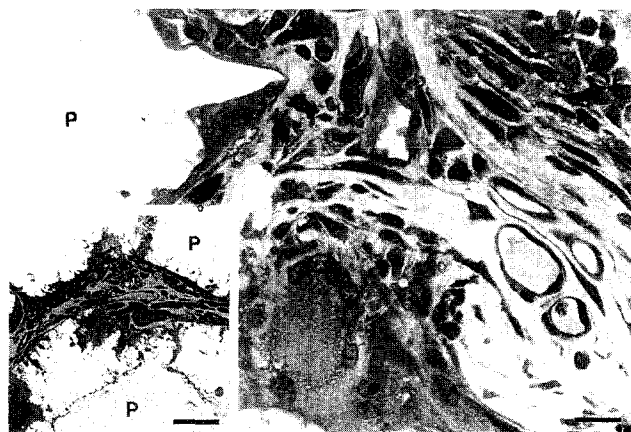


Fig. 23. Copolymer 80 (P) after 10 weeks of implantation. Many foreign body giant cells can be observed in an attempt to engulf the polymer debris. The bar represents 15  $\mu\text{m}$  and 40  $\mu\text{m}$  (insert).

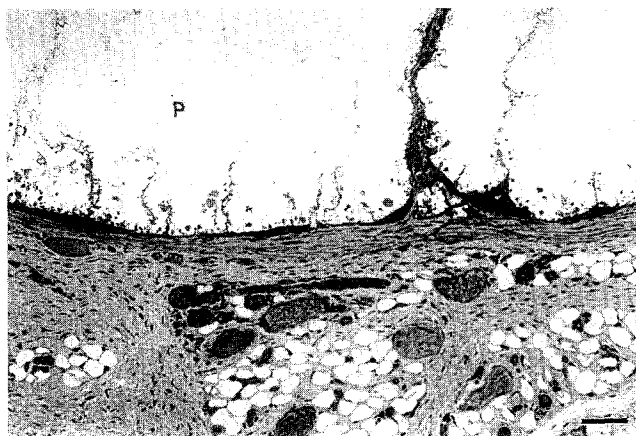


Fig. 24. Polymer 100 (P) after 10 weeks of implantation. Only minor phagocytic activity can be observed. The bar represents 63  $\mu\text{m}$ .

Before this point is reached, fragments of the polymer bulk become loosened or are internalized by macrophages or giant cells which, in turn, continue with the degradation process.

The process ends when all foreign material is hydrolyzed and phagocytosed. Then the stimulus for cellular activity disappears and only some scar tissue remains.

To compare these observations with the degradation behaviour of other polymers it is relevant to pay some attention to DL-lactide and glycolic acid/lactic acid copolymers in different compositions, as studied by others (see also Table 4). Pure poly (DL-lactide), as studied by Visscher *et al.*<sup>93</sup> with a molecular weight ( $M_w$ ) of 38900, demonstrated a similar foreign body reaction, although a somewhat longer degradation time (420 days) was observed in this intramuscular model. Visscher *et al.*, however, describe the reaction as being a totally non-adverse tissue reaction with an initial mild subacute myositis which subsides to a minimal localized connective tissue and foreign body reaction.

Pitt *et al.*<sup>40</sup> recognized that the decrease in molecular weight of DL-lactide and caprolactone

was accompanied by an increase in crystallinity and brittleness, but weight loss was not observed until a limiting  $M_n$  of approximately 5000 was reached. Furthermore, Pitt *et al.* stated that the mechanism of degradation was non-enzymatic random hydrolytic ester bond cleavage, and the time interval needed for complete degradation is determined by the initial molecular weight and the chemical structure of the implant.

Zaikov<sup>99</sup> describes a similar degradation mechanism for polyglycolic acid. Considerably shorter degradation times are observed. Polyglycolic acid shows a very severe macrophage mediated tissue response.<sup>29,100</sup>

It seems logical that copolymers composed of DL-lactide and glycolic acid residues would demonstrate an in-between degradation time but a similar tissue response and degradation mechanism, as compared to the homopolymers. Many investigators have studied this promising copolymer. In a clinical situation, Bowald and coworkers implanted polyglactin 910 suture material woven into a vascular prosthesis.<sup>94,95</sup> Polyglactin 910 (Vicryl, Ethicon) is composed of 90% glycolic acid and 10% DL-lactic acid residues; molecular weights were not mentioned. They observed a degradation of the graft within 40 days after implantation *in situ*. Smooth muscle cells and fibroblasts infiltrated the graft. Foreign body giant cells and a few inflammatory cells were observed around residual polyglactin 910 filaments. Capillaries were observed inside the grafts and collagenous tissue had formed.

The same copolymer was studied by Matlaga and Salthouse<sup>10</sup> focusing on the ultrastructure of tissue cells reacting to the copolymer. They observed a degradation time of approximately 2 months after intramuscular implantation in rats. The polyglactin 910 evoked a foreign body reaction, mediated by macrophages. The initial cellular reactions against the biomaterial (the first 4 weeks) were quite similar

Table 4. Lactic Acid/Glycolic Acid Copolymers: Chemical Composition and Molecular Weight

Basic polymer	% Copolymers	$M_w$	Approximate degradation time (days)	ref. no
Poly L-lactic acid	—	38900	420	93
Poly DL-lactic acid	—	14000 ( $M_n$ )	200	40
		49000 ( $M_n$ )	420	
Poly DL-lactic acid	10% glycolic acid	—	> 60	10, 94, 95
Poly DL-lactic acid	20% glycolic acid	40000	90–120	96
Poly DL-lactic acid	50% glycolic acid	40000	< 60	97, 98

as described for poly L-lactide: a wound healing reaction followed by an encapsulation by macrophages and the formation of a collagen forming fibrous layer.<sup>101</sup>

From day 35 onwards, significant degradation of the implant was observed. The amount of giant cells and their activity seemed even higher, as judged by the abundance of mitochondria, lysosomes, and high levels of acid phosphatase activity. Matlaga and Salthouse suggest that the ultimate degradation products, lactic and glycolic acid, were metabolized within giant cells and macrophages. The site of metabolism is believed to be within the mitochondria via the Krebs cycle enzymes.<sup>13</sup>

Kitakado *et al.*<sup>96</sup> developed a poly DL-lactic acid/glycolic acid copolymer containing 80% lactic acid and with a molecular weight of 40000 ( $M_w$ ). The degradation time varied between 3 and 4 months. Little foreign body reaction was described.

Regarding the DL-lactic acid and glycolic acid polymers and copolymers, the degradation rate influences the degree of foreign body reaction. In turn, the degradation rate will be determined by the chemical nature and molecular weight of the polymers. The more DL-lactic acid present in the copolymer, the lower the degradation rate, resulting in a less severe foreign body reaction.

Consequently, the degree of foreign body reaction anticipated after implantation of 50/50 polylactic/polyglycolic acid is even more severe than that of 80/20 and 90/10. The degradation time will also be shorter. Visscher *et al.*<sup>97,98</sup> studied the intramuscular degradation of 50/50 poly(DL-dilactide-co-glycolide) microcapsules. Complete degradation was observed within 60 days. Many foreign body giant cells were observed around the implants, although the authors preferred to refer to the reaction as 'minimal foreign body response'.

The mechanism of degradation was suggested to be hydrolysis of ester bonds. Hydrolysis of the microcapsules was first observed within the bulk of the material. The erosion of internal matrix before breakdown of the external layer may indicate a difference in the crystalline structure of the outer shell. Vert and coworkers<sup>102</sup> also described this phenomenon of initial internal degradation.

It may be clear that the biodegradability of copolymers of lactic acid and glycine or glycolic acid is influenced by their chemical composition and molecular weight. Additives such as plasticizers, initiators, solvents, monomer residues, ion-diffusion, ionic strength of surrounding fluid, etc., can also influence the biocompatibility and biodegradability of the (co)polymers.<sup>102-106</sup>

A factor still not mentioned is the enzymatic breakdown of polymer materials.<sup>11</sup> Williams and coworkers have described the effects of enzymes on polyglycolic acid,<sup>107</sup> polylactic acid<sup>108</sup> and even on nylon, PMMA and polyethyleneterephthalate (PET).<sup>109</sup> Polyglycolic acid was said to be susceptible to bromelain, esterase and leucine aminopeptidase activity, which enhanced the hydrolysis. Polylactic acid was susceptible to bromelain, pronase and proteinase K. Even some nominally stable polymers, such as polyethyleneterephthalate and nylon 66<sup>109</sup> were affected by enzymes (e.g. papain). On the other hand, other authors demonstrated that in-vivo implantation studies of poly(L-lactic acid) showed no influence of enzymes.<sup>97,110</sup> Schakenraad *et al.*<sup>110</sup> and Pitt *et al.*<sup>40</sup> did not observe significant differences between in-vivo and in-vitro degradation studies, suggesting no influence of enzymes on the rate of hydrolysis. Williams<sup>11</sup> however did demonstrate an effect of enzymes on hydrolysis, both *in vitro* and *in vivo*. The concentration of enzymes at the polymer surface *in vivo* however can not accurately be determined nor influenced and is probably much lower than the level in in-vitro studies.

These considerations made us conclude that enzymatic enhancement of hydrolysis will probably not play a role of importance with in-vivo degradation of aliphatic polyesters.

In conclusion, the in-vitro and in-vivo degradation patterns of polylactic acid, polyglycolic acid, polyglycine and their copolymers are quite similar. Bulk hydrolysis of ester bonds is the most apparent means of degradation. The initial molecular weight and the chemical composition (e.g. ratio of copolymers) determines the ultimate degradation time and degree of tissue reaction. The biocompatibility of all these polymers and copolymers is acceptable, since the monomer is a natural residue; however, a change in molecular weight and thus the start of solubility determines the onset of a severe foreign body reaction, mediated by active macrophages and foreign body giant cells. In itself this reaction is not considered to be an indication of poor biocompatibility but merely a part of the foreign body 'healing' reaction. The cascade of events is focused on removing the soluble fragments and the small solid fragments from the scene of action.

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